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Flupirtine Modulates both KCNQ K^+ Channels and $GABA_A$ Receptors in Hippocampal Neurons

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KCNQ/M-channels are slowly gating and non-inactivating K^+ channels. They are widely expressed in the nervous system and play major roles in regulation of neuronal excitability. Still controversial is the functional role of M-channels in neurotransmitter release. To clarify this issue, we sought to test drugs known to activate or inhibit M-channels for their effects on transmitter release or synaptic transmission, using hippocampal ''micro-island'' autapses or mass cultures. A possible confounding issue are effects of the drugs on excitatory or inhibitory inotropic receptors. Indeed, flupirtine, a drug with poorly understood mechanisms of action had no effect on glutamatergic, but prolonged GABAergic, autaptic currents. In mass cultures, we found direct, but bi-phasic, effects of flupirtine on postsynaptic $GABA_A$ receptors ($GABA_AR$), in which flupirtine (30 μM) reduced by 3-fold the EC_{50} value for GABA-induced currents but reduced the maximal current by 15%. At 100 μM, flupirtine induced an inward current that was $GABA_AR$-mediated, since it was abolished by bicuculline (30 ∈ μM). To differentiate between effects on synaptic and extrasynaptic $GABA_AR$, miniature inhibitory postsynaptic currents (mIPSCs) were analyzed. Flupirtine did not alter the rise time, decay time, nor amplitude of the mIPSCs, but enhanced the bicuculline-sensitive tonic current. When synaptic $GABA_ARs$ were blocked with penicillin G (5 mM), flupirtine potentiated GABA-induced currents, enhancing maximal amplitudes by 43%. These results indicate that flupirtine potentiates GABAergic transmission by distinct effects on synaptic and extrasynaptic $GABA_AR$ subtypes. We are investigating the role of presynaptic KCNQ channels on neurotransmitter release using other KCNQ openers, such as retigabine, or the blockers linopirdine and XE991. To ask if M-type channels localize to hippocampal pre-synaptic terminals, we will perform co-immunostaining of KCNQ subunits and presynaptic markers (e.g. synaptophysin) via confocal microscopy. Supported by NIH and the Austrian Science Fund.

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Incontinent Pool of Primed Vesicles in Synaptotagmin-1-Deficient GABAergic Synapses

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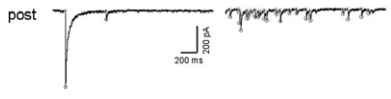
The coupling of neuronal excitation to release of chemical transmitter at synaptic endings is mediated by an electrically gated entry of Ca2+-ions that within a fraction of a millisecond leads to exocytotic fusion of vesicles with the presynaptic membrane. In this rapid process, members of the synaptotagmin (Syt) family of vesicular membrane proteins are likely to function as the Ca2+-sensing molecular trigger. The trigger mechanism employed, however, is currently under debate. Here, using high resolution dual simultaneous pre- and postsynaptic whole cell patch clamp recordings from Syt1-deficient GABAergic neurons, we report a strong potentiation of asynchronous release in the absence of synchronous release. In addition, we show that Syt1-deficient vesicles are hypersensitive to Ca2+-independent osmotic stimulation of exocytosis and that, unlike in controls, chemical priming into a highly fusion competent state directly results in their spontaneous exocytosis. Therefore, the primed vesicle pool is incontinent in these synapses. These findings argue strongly that an inhibitory, latch-like action by Ca2+-free Syt1 on SNARE-function is part of its physiological role in the mammalian nervous system to ensure transmitter release closely timed to the presynaptic action potential.



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Modulation of the Excitatory Synaptic Transmission in Isolated Rat Hippocampus by Direct Current Stimulation

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Transcranial direct current stimulation (DCS) is a noninvasive method for changing cortical excitability. When applied to the scalp over the human cerebral cortex, a cathodal direct current stimulating electrode induces a lasting decrease in excitability, whereas an anodal stimulating electrode enhances excitability. However, mechanisms of the DCS effect at cellular and molecular levels are not well understood. To elucidate the DCS mechanisms, we developed methods for DCS in isolated hippocampal slices and tested the effects of current magnitude and polarity on the CA1 field excitatory post-synaptic potential (fEPSP). METHODS: 400 ∈ μm hippocampal slices were prepared from rat pups (P18 - P24). Baseline CA1 fEPSP slope and amplitude were obtained by stimulation of the Schaffer collateral fibers while recording from the CA1 region. DCS (range: −400 to +400 ∈ μA) was applied for 5 minutes via two silver-chloride pellet electrodes located outside of the slice and 1 mm away from CA1 and CA3 regions, respectively. RESULTS: The fEPSPs persisted during DCS (−400 to +200 ∈ μA) and were modulated by current amplitude and polarity. Maximal reduction (by 30%) of fEPSPs amplitude and slope by DCS was achieved with 400 ∈ μA DC directed toward the CA1 region (−400 ∈ μA). Maximal facilitation of fEPSPs (by 18%) was achieved with 100∈ μA DC in opposite direction. The dependence of fEPSP amplitude on DCS amplitude was non-linear with the maximum of the first derivative at 0 ∈ μA. DISCUSSION: We demonstrate for the first time that (1) external DCS can modulate EPSP amplitude and slope, and (2) that the magnitude and direction of the EPSP change is dependent on the magnitude and direction of the direct current. Our data support the hypothesis that DCS effect on EPSPs is due to polarization of the postsynaptic membrane.

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How Does Synaptic Dynamics Regulate Neuronal Encoding?

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Neuronal spikes and synaptic signals are presumably basic codes in the brain to control behaviors. In terms of their interaction, spike patterns influence synaptic plasticity via presynaptic mechanisms. It is not documented how synaptic dynamics regulate neuronal encodings, especially their quantitative correlations, which we studied at cortical circuits assembled from pyramidal and GABAergic neurons. Our studies at unitary synapses demonstrate that postsynaptic responses are constancy over time, e.g., glutamate receptor-channel currents at GABAergic cells and glutamate transport currents at astrocytes, indicating quantal glutamate release. Moreover, sequential presynaptic spikes up-regulate glutamate release probability and synaptic strength in a linearly correlation. Spike frequency and presynaptic Ca^{2+} levels mediate this increment of release probability, as well as raise the efficiency of probability vs. synaptic facilitation. In terms of their physiological significance, an increase of glutamate release probability improves spike timing precision and capacity at postsynaptic neurons, and the signals integrated from quantal glutamatergic synapses drive spike encodings at postsynaptic neurons to be precisely reliable, which regulates spike encodings at pyramidal cells to be precise via a feedback inhibition. Therefore, the potentiality in releasing presynaptic glutamates and the patterns of quantal release are beneficial to signal transmission at the synapses and spike encodings at the neuron in neural circuits for editing homeostatic brain softwares to manage well-organized behaviors.

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pH-Sensitive Fluorescent Lipids as Novel Probes to Monitor Vesicle Recycling

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During synaptic transmission, neurotransmitters stored in presynaptic vesicles are released by excocytosis through fusion of vesicles with the plasma membrane. In a subsequent step, membranes and proteins at the synapse are reinternalized by a reverse process, endocytosis. In the analysis of this synaptic vesicle cycle genetically encoded pH-sensitive fluorescent proteins like the GFP-derivative pHluorin have become indispensable tools. These probes are capable of detecting changes in pH that accompany excocytosis and subsequent reacidification of endocytosed vesicles. Here we describe a new class of fluorescent probes, based on pH-sensitive organic dyes coupled to phospholipids, as promising alternative to genetically encoded fluorescent proteins like pHluorin. Moreover the pH-dependent fluorescence properties of these dyes are opposite to those of pHluorin.

In hippocampal neurons, cell membranes can be stained in a pH dependent manner, and upon quenching of the fluorescence at the plasma membrane by a slightly basic pH, vesicle recycling can be monitored yielding fluorescence transients with kinetics mirroring those of the well characterized pHluorin signal. Furthermore, this approach can be used to study vesicle recycling in acute preparations like bipolar cells of the retina, where application of genetically encoded probes was not possible so far.

This experimental approach using pH-dependent fluorescent lipids has not only the potential of being used in a variety of cellular and slice preparations, but in addition will shed light on an important presynaptic mechanism neglected so far, namely lipid recycling. Comparison of vesicle incorporation of different